



## CheKine™ Micro Soil Leucine Arylamidase (S-LAP) Activity Assay Kit

Cat #: KTB4020

Size: 96 T

	<b>Micro Soil Leucine Arylamidase (S-LAP) Activity Assay Kit</b>		
<b>REF</b>	<b>Cat #:</b> KTB4020	<b>LOT</b>	<b>Lot #:</b> Refer to product label
	<b>Applicable samples:</b> Soil		
	<b>Storage:</b> Stored at 4°C for 12 months, protected from light		

### Assay Principle

Soil Leucine Arylamidase (LAP) can hydrolyze enzyme with N-terminal leucine at the peptide chain, and was secreted by soil microorganism. The S-LAP activity changes are closely related to certain pathological states of the body. CheKine™ Micro Soil Leucine Arylamidase (S-LAP) Activity Assay Kit provides a simple, convenient and rapid Soil LAP activity detection method. The detection principle is that the p-nitroaniline produced by S-LAP hydrolysis of L-leucyl-p-nitroaniline, there is a characteristic absorption peak at 405 nm. The S-LAP activity is calculated by measuring of absorbance changes.

### Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
Reagent I	70 mL	4°C
Reagent II	1	4°C, protected from light

### Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 405 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Centrifuge, water bath
- Deionized water

### Reagent Preparation

**Reagent I** : Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

**Reagent II** : For 96 T, add 30 mL Reagent I before use; Fully dissolve it for use (when it is difficult to dissolve, it can be heated in a 60°C water bath for 30 min to promote dissolution), aliquoted and stored at -20°C. Avoid repeated freezing and thawing.

### Sample Preparation

Fresh soil was recommended

## Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 405 nm, visible spectrophotometer was returned to zero with deionized water.
2. Enzymatic reaction (the following operations are performed in a 1.5 mL centrifuge tube).

Reagent	Test Tube (μL)	Control Tube (μL)
Sample (mg)	50	50
Reagent I	0	300
Reagent II	300	0

Mix well, put it in a water bath at 37°C shaking for 1 h, centrifuge at 8,000 g at 4°C for 10 min, and take 200 μL supernatant and add into 96-well plate or microglass cuvette. The absorbance value was determined by 405 nm, which was recorded as  $A_{\text{Test}}$  and  $A_{\text{Control}}$ , calculated  $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Control}}$ . One control tube was suggested for each test.

**Note:** In order to guarantee the accuracy of experimental results, pre-experiments were suggested using 2-3 samples with potential significant differences. Increase the sample quantity appropriately. If  $\Delta A_{\text{Test}}$  is greater than 1.0, the sample can be appropriately diluted, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

## Data Analysis

**Note:** We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well plates calculation formula

Definition of unit: 1 nmol of p-nitroaniline produced per gram soil per minute is defined as an enzyme activity unit.

Calculated S-LAP activity using microcuvette:

$$\text{S-LAP (U/g sample)} = \Delta A \times V_{\text{Total reaction}} \div (\epsilon \times d) \times 10^9 \div W \div T = \mathbf{1.013 \times \Delta A \div W}$$

$V_{\text{Total reaction}}$ : Total volume of reaction system,  $3 \times 10^{-4}$  L;  $\epsilon$ : molar extinction coefficients of p-nitroaniline,  $9.87 \times 10^3$  L/mol/cm;  $d$ : 96-well plate optical diameter, 0.5 cm;  $T$ : Reaction time, 1 h=60 min;  $W$ : Sample weight, g;  $10^9$ : coefficients, 1 mol=1×10<sup>9</sup> nmol.

B. Microglass cuvette calculation formula

The optical diameter  $d$ :0.5 cm in the above calculation formula can be adjusted to  $d$ :1 cm for calculation.

## Typical Data

Example 1: Take 0.05 g fresh soil and S-LAP activity was detected in 96-well plate, the resulting  $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Control}} = 0.266 - 0.053 = 0.213$ , the S-LAP activity in this soil sample (U/g) =  $1.013 \times 0.213 \div 0.05 = 4.32$  U/g.

## Recommended Products

Catalog No.	Product Name
KTB3040	CheKine™ Micro Glutamate Synthase (GOGAT) Assay Kit
KTB3041	CheKine™ Micro Glutamic Acid Dehydrogenase (GDH) Assay Kit

## Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.